(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 085 773 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
18.03.2020 Bulletin 2020/12

(21) Application number: **16164746.6**

(22) Date of filing: **01.06.2009**

(51) Int Cl.:
C12N 5/16 *(2006.01)* ***C07K 16/00*** *(2006.01)*
C07K 16/24 *(2006.01)* ***C07K 14/545*** *(2006.01)*
G01N 33/68 *(2006.01)* ***A61K 39/395*** *(2006.01)*

(54) USES OF IL-1 ALPHA ANTIBODIES

VERWENDUNG VOM IL-1-ALPHA-ANTIKÖRPERN

UTILISATION D'ANTICORPS IL-1 ALPHA

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK TR
Designated Extension States:
AL BA RS

(30) Priority: **30.05.2008 US 57586 P**
10.12.2008 US 121391 P
14.05.2009 US 178350 P

(43) Date of publication of application:
26.10.2016 Bulletin 2016/43

(60) Divisional application:
20156323.6

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
09758739.8 / 2 285 409

(73) Proprietor: **XBiotech, Inc**
Vancouver, British Columbia V6E 2E9 (CA)

(72) Inventor: **SIMARD, John**
Austin, TX 78744 (US)

(74) Representative: **Duffield, Stephen**
Carpmaels & Ransford LLP
One Southampton Row
London WC1B 5HA (GB)

(56) References cited:
US-A- 5 959 085

- **GARRONE P ET AL: "Generation and characterization of a human monoclonal autoantibody that acts as a high affinity interleukin-1 alpha specific inhibitor", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 33, no. 7-8, 1 May 1996 (1996-05-01), pages 649-658, XP002410040, ISSN: 0161-5890, DOI: 10.1016/0161-5890(96)00017-X**
- **WOOD D D ET AL: "RELEASE OF INTERLEUKIN-1 FROM HUMAN SYNOVIAL TISSUE IN-VITRO", ARTHRITIS AND RHEUMATISM, vol. 28, no. 8, 1985, pages 853-862, XP002759448, ISSN: 0004-3591**
- **VAZQUEZ A ET AL: "Interleukin 1 can replace monocytes for the specific human B-cell response to a particulate antigen", CELLULAR IMMUNOLOGY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 86, no. 2, 1 July 1984 (1984-07-01), pages 287-298, XP024003547, ISSN: 0008-8749, DOI: 10.1016/0008-8749(84)90383-6 [retrieved on 1984-07-01]**
- **MUSTEA A ET AL: "Decreased IL-1 RA concentration in ascites is associated with a significant improvement in overall survival in ovarian cancer", CYTOKINE, ACADEMIC PRESS LTD, PHILADELPHIA, PA, US, vol. 42, no. 1, 1 April 2008 (2008-04-01), pages 77-84, XP022603032, ISSN: 1043-4666, DOI: 10.1016/J.CYTO.2008.01.011 [retrieved on 2008-03-07]**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- **Anastasios J Karayiannakis ET AL: "Serum leptin levels and their response during laparoscopic and open cholecystectomy", European cytokine network, 1 January 2005 (2005-01-01), page 91, XP055415175, France Retrieved from the Internet: URL:http://www.jle.com/download/ecn-266023-serum_leptin_levels_and_their_response_during_laparoscopic_and_open_cholecystectomy--Wd9nRH8AAQEAAG4KfEUAAAAK-a.pdf [retrieved on 2017-10-13]**

Description

FIELD OF THE INVENTION

[0001] The disclosure relates generally to the fields of immunology, inflammation, cancer, vascular disorders, and medicine. More particularly, the disclosure relates to antibodies (Abs) which specifically bind interleukin-la (IL-1α) and methods of using such Abs to treat, prevent, or detect a pathology associated with aberrant IL-1α expression. The invention relates to a method for quantifying the amount of IL-1α in a biological sample.

BACKGROUND

[0002] IL-1α is pro-inflammatory cytokine that plays a role in a number of different activities including inflammation, immune responses, tumor metastasis, and hematopoiesis. IgG autoantibodies against IL-1α occur naturally in the general human population and are thought to be beneficial in diseases such as atherosclerosis.

[0003] US5959085 describes the use of antibodies capable of specifically binding IL-1α for the immunoprecipitation of the target antigen. Wood et al (Arthritis and Rheumatism (1985) 28(2):853-862) describe the enrichment and isolation of IL-1α from a biological sample, including size exclusion filtration.

SUMMARY

[0004] The disclosure is based on the development of fully human monoclonal Abs (mAbs) that include (i) an antigen-binding variable region that exhibits very high binding affinity for human IL-1α and (ii) a constant region that is effective at both activating the complement system though C1q binding and binding to several different Fc receptors. The IL-1α specific mAbs described herein was made by replacing the constant region of a human IgG4 mAb having a variable region specific for human IL-1α with the constant region of a human IgG1 mAb.

[0005] Accordingly, the disclosure features a purified human IgG1 mAb that specifically binds to human IL-1α, the mAb including a heavy chain covalently joined to a light chain. The heavy chain can include the amino acid sequence of SEQ ID NO: 9 and the light chain can include the amino acid sequence of SEQ ID NO:11.

[0006] Also within the disclosure is a set of isolated nucleic acids including a first nucleic acid encoding the heavy chain of a human IgG1 mAb that specifically binds to IL-1α, and a second nucleic acid encoding the light chain of the human IgG1 mAb that specifically binds to human IL-1α. The first nucleic acid can encode the amino acid sequence of SEQ ID NO: 9 and the second nucleic acid can encode the amino acid sequence of SEQ ID NO:11. The first nucleic acid can include the nucleotide sequence of SEQ ID NO: 10 and the second nucleic acid can include the nucleotide sequence of SEQ ID NO:12.

[0007] In another aspect, the invention features an expression vector including a nucleic acid encoding the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[0008] Another feature of the disclosure is an isolated host cell (e.g. a mammalian cell such as a CHO cell) including set of isolated nucleic acids including a first nucleic acid encoding the heavy chain of a human IgG1 mAb that specifically binds to IL-1α, and a second nucleic acid encoding the light chain of the human IgG1 mAb that specifically binds to human IL-1α. The heavy chain can include the amino acid sequence of SEQ ID NO: 9 and a light chain can include the amino acid sequence of SEQ ID NO:11.

[0009] The disclosure further features a method of killing a cell expressing human IL-1α. This method can include the step of contacting the cell with a purified human IgG1 mAb that specifically binds to human IL-1α.

[0010] A method of inhibiting migration of a human cell through a basement membrane matrix is also within the disclosure. This method can include the step of adding a purified mAb that specifically binds to human IL-1α to a mixture including a basement membrane matrix and the human cell.

[0011] Further within the disclosure is a method of inhibiting an IL-1α-induced increase in ICAM-1 and/or E-selectin expression on the surface of a human endothelial cell. This method can include the step of adding a purified mAb that specifically binds to human IL-1α to a mixture including the endothelial cell and IL-1α.

[0012] The disclosure additionally includes a method of tracking inflammation in a human subject previously subjected to the steps of: obtaining from the subject a first sample of peripheral blood mononuclear cells at a first time; contacting the first sample with a purified mAb that specifically binds to human IL-1α; and determining the percent of cells in the first sample that bind the monoclonal Ab. This method can include the steps of: (a) obtaining from the subject a second sample of peripheral blood mononuclear cells at a second time; (b) contacting the second sample with the purified mAb that specifically binds to human IL-1α; (c) determining the percent of cells in the second sample that bind the monoclonal Ab; and (d) comparing the percent of cells in the first sample that bind the mAb to the percent of cells in the second sample that bind the monoclonal Ab.

[0013] In the foregoing methods, the purified mAb can be a human IgG1 mAb including a heavy chain covalently joined

to a light chain, e.g., wherein the heavy chain includes the amino acid sequence of SEQ ID NO: 9 and the light chain includes the amino acid sequence of SEQ ID NO:11.

[0014] The method of the invention features the steps of: (a) enriching a biological sample obtained from a human subject using a filter to separate molecules according to molecular weight into a first fraction including intact IgG complexed with IL-1α and second fraction including molecules less than 100 Kda; and (b) quantifying the amount of IL-1α in the first fraction.

[0015] Another method within the invention features the steps of: (a) enriching a sample of plasma obtained from a human subject using a filter that separates molecules according to molecular weight into a first fraction including intact IgG complexed with IL-1α and second fraction including molecule less than 100 Kda; (b) adding the first fraction to a substrate including immobilized anti-human IgG Abs under conditions that allow IgG in the first fraction to specifically bind the anti-human IgG Abs immobilized on the substrate; (c) washing the substrate to remove material in the first fraction that does not specifically bind the immobilized anti-human IgG Abs; (d) contacting the substrate washed in step (c) with an Ab that specifically binds human IL-1α under conditions that allows the Ab that specifically binds human IL-1α to specifically bind any human IL-1α bound to the substrate; (e) washing the substrate to remove any of the Ab that specifically binds human IL-1α that is not bound to the substrate; and (f) quantifying the amount of Ab that specifically binds human IL-1α remaining bound to the substrate after step (e).

[0016] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Commonly understood definitions of biological terms can be found in Rieger et al., Glossary of Genetics: Classical and Molecular, 5th edition, Springer-Verlag: New York, 1991; and Lewin, Genes V, Oxford University Press: New York, 1994.

[0017] The term "specifically binds", as used herein, when referring to a polypeptide (including Abs) or receptor, refers to a binding reaction which is determinative of the presence of the protein or polypeptide or receptor in a heterogeneous population of proteins and other biologics. Thus, under designated conditions (e.g. immunoassay conditions in the case of an Ab), the specified ligand or Ab binds to its particular "target" and does not bind in a significant amount to other proteins present in the sample or to other proteins to which the ligand or Ab may come in contact in an organism. Generally, a first molecule that "specifically binds" a second molecule has an equilibrium affinity constant greater than about 105 (e.g., 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, and 10^{12} or more) liters/mole for that second molecule.

[0018] When referring to a protein molecule such as an Ab, "purified" means separated from components that naturally accompany such molecules. Typically, an Ab or protein is purified when it is at least about 10% (*e.g.,* 9%, 10%, 20%, 30% 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, 99.9%, and 100%), by weight, free from the non-Ab proteins or other naturally-occurring organic molecules with which it is naturally associated. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. A chemically-synthesized protein or other recombinant protein produced in a cell type other than the cell type in which it naturally occurs is "purified."

[0019] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In the case of conflict, the present specification, including definitions will control. In addition, the particular embodiments discussed below are illustrative only and not intended to be limiting.

DETAILED DESCRIPTION

[0020] The invention relates to a method for quantifying the amount of IL-1α in a biological sample, the method comprising the steps of:

(a) separating a biological sample obtained from a human subject using a size-exclusion filter that separates molecules according to molecular weight into a first fraction comprising intact IgG complexed with IL-1α and second fraction consisting of molecules less than 100 Kda, wherein the biological sample comprises intact IgG complexed with IL-1α, wherein the first fraction does not pass through the filter, and wherein the second fraction passes through the filter; and

(b) quantifying the amount of IL-1α in the first fraction.

[0021] Preferably, the method comprises

(a) separating a biological sample obtained from a human subject using a size-exclusion filter that separates molecules according to molecular weight into a first fraction comprising intact IgG complexed with IL-1α and second fraction consisting of molecules less than 100 Kda, wherein the biological sample comprises intact IgG complexed with IL-1α, wherein the first fraction does not pass through the filter, and wherein the second fraction passes through the filter;

(b) adding the first fraction to a substrate comprising immobilized anti-human IgG antibodies under conditions that allow IgG in the first fraction to specifically bind the anti-human IgG antibodies immobilized on the substrate;
(c) washing the substrate to remove material in the first fraction that does not specifically bind the immobilized anti-human IgG antibodies;
(d) contacting the substrate washed in step (c) with an antibody that specifically binds human IL-1α under conditions that allows the antibody that specifically binds human IL-1α to specifically bind any human IL-1α bound to the substrate;
(e) washing the substrate to remove any of the Ab that specifically binds human IL-1α that is not bound to the substrate; and
(f) quantifying the amount of Ab that specifically binds human IL-1α remaining bound to the substrate after step (e).

[0022] The disclosure describes compositions and methods relating to fully human mAbs that include (i) an antigen-binding variable region that exhibits very high binding affinity for IL-1α and (ii) a constant region that is effective at both activating the complement system though C1q binding and binding to several different Fc receptors. The below described preferred embodiments illustrate adaptation of these compositions and methods. Nonetheless, from the description of these embodiments, other aspects of the disclosure can be made and/or practiced based on the description provided below.

[0023] Methods involving conventional immunological and molecular biological techniques are described herein. Immunological methods (for example, assays for detection and localization of antigen-Ab complexes, immunoprecipitation, immunoblotting, and the like) are generally known in the art and described in methodology treatises such as Current Protocols in Immunology, Coligan et al., ed., John Wiley & Sons, New York. Techniques of molecular biology are described in detail in treatises such as Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Sambrook et al., ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and Current Protocols in Molecular Biology, Ausubel et al., ed., Greene Publishing and Wiley-Interscience, New York. Ab methods are described in Handbook of Therapeutic Abs, Dubel, S., ed., Wiley-VCH, 2007. Cell culture techniques are generally known in the art and are described in detail in methodology treatises such as Culture of Animal Cells: A Manual of Basic Technique, 4th edition, by R Ian Freshney, Wiley-Liss, Hoboken, N.J., 2000; and General Techniques of Cell Culture, by Maureen A Harrison and Ian F Rae, Cambridge University Press, Cambridge, UK, 1994. Methods of protein purification are discussed in Guide to Protein Purification: Methods in Enzymology, Vol. 182, Deutscher M P, ed., Academic Press, San Diego, Calif., 1990.

[0024] The disclosure describes a fully human mAb that includes (i) an antigen-binding variable region that exhibits very high binding affinity for human IL-1α and (ii) a constant region that is effective at both activating the complement system though C1q binding and binding to several different Fc receptors. The human Ab is preferably an IgG1. The Ka of the Ab is preferably at least 1 $x10^9$ M^{-1} or greater (e.g., greater than 9 $x10^{10}$ M^{-1}, 8 $x10^{10}$ M^{-1}, 7 $x10^{10}$ M^{-1}, 6 $x10^{10}$ M^{-1}, 5 $x10^{10}$ M^{-1}, 4 $x10^{10}$ M^{-1}, 3 $x10^{10}$ M^{-1}, 2 $x10^{10}$ M^{-1}, or 1 $x10^{10}$ M^{-1}).

[0025] Because B lymphocytes which express Ig specific for human IL-1α occur naturally in human beings, a presently preferred method for raising mAbs is to first isolate such a B lymphocyte from a subject and then immortalize it so that it can be continuously replicated in culture. Subjects lacking large numbers of naturally occurring B lymphocytes which express Ig specific for human IL-1α may be immunized with one or more human IL-1α antigens to increase the number of such B lymphocytes. Human mAbs are prepared by immortalizing a human Ab secreting cell (e.g., a human plasma cell). See, e.g., U.S. patent no. 4,634,664.

[0026] In an exemplary method, one or more (e.g., 5, 10, 25, 50, 100, 1000, or more) human subjects (e.g., subjects not previously administered a human IL-1α vaccine) are screened for the presence of such human IL-1α-specific Ab in their blood. Those subjects that express the desired Ab can then be used as B lymphocyte donors. In one possible method, peripheral blood is obtained from a human donor that possesses B lymphocytes that express human IL-1α-specific Ab. Such B lymphocytes are then isolated from the blood sample, e.g., by cells sorting (e.g., fluorescence activated cell sorting, "FACS"; or magnetic bead cell sorting) to select B lymphocytes expressing human IL-1α-specific Ig. These cells can then be immortalized by viral transformation (e.g., using EBV) or by fusion to another immortalized cell such as a human myeloma according to known techniques. The B lymphocytes within this population that express Ig specific for human IL-1α can then be isolated by limiting dilution methods (e.g., cells in wells of a microtiter plate that are positive for Ig specific for human IL-1α are selected and subcultured, and the process repeated until a desired clonal line can be isolated). See, e.g., Goding, Monoclonal Abs: Principles and Practice, pp. 59-103, Academic Press, 1986. Those clonal cell lines that express Ig having at least nanomolar or picomolar binding affinities for human IL-1α are preferred. MAbs secreted by these clonal cell lines can be purified from the culture medium or a bodily fluid (e.g., ascites) by conventional Ig purification procedures such as salt cuts, size exclusion, ion exchange separation, and affinity chromatography.

[0027] Although immortalized B lymphocytes might be used in *in vitro* cultures to directly produce mAbs, in certain cases it might be desirable to use heterologous expression systems to produce mAbs. See, e.g., the methods described

in U.S. patent application number 11/754,899. For example, the genes encoding an mAb specific for human IL-1α might be cloned and introduced into an expression vector (e.g., a plasmid-based expression vector) for expression in a heterologous host cell (e.g., CHO cells, COS cells, myeloma cells, and E. coli cells). Because Igs include heavy (H) and light (L) chains in an H_2L_2 configuration, the genes encoding each may be separately isolated and expressed in different vectors.

[0028] Although generally less preferred, chimeric mAbs (e.g., "humanized" mAbs), which are antigen-binding molecules having different portions derived from different animal species (e.g., variable region of a mouse Ig fused to the constant region of a human Ig), might be used in the invention. Such chimeric Abs can be prepared by methods known in the art. E.G., Morrison et al., Proc. Nat'l. Acad. Sci. USA, 81:6851, 1984; Neuberger et al., Nature, 312:604, 1984; Takeda et al., Nature, 314:452, 1984. Similarly, Abs can be humanized by methods known in the art. For example, monoclonal Abs with a desired binding specificity can be commercially humanized or as described in U.S. Pat. Nos. 5,693,762; 5,530,101; or 5,585,089.

[0029] The mAbs described herein might be affinity matured to enhance or otherwise alter their binding specificity by known methods such as VH and VL domain shuffling (Marks et al. Bio/Technology 10:779-783, 1992), random mutagenesis of the hypervariable regions (HVRs) and/or framework residues (Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813, 1994; Schier et al. Gene 169:147-155, 1995; Yelton et al. J. Immunol. 155:1994-2004, 1995; Jackson et al., J. Immunol. 154(7):3310-9, 1995; and Hawkins et al, J. Mol. Biol. 226:889-896, 1992. Amino acid sequence variants of an Ab may be prepared by introducing appropriate changes into the nucleotide sequence encoding the Ab. In addition, modifications to nucleic acid sequences encoding mAbs might be altered (e.g., without changing the amino acid sequence of the mAb) for enhancing production of the mAb in certain expression systems (e.g., intron elimination and/or codon optimization for a given expression system). The mAbs described herein can also be modified by conjugation to another protein (e.g., another mAb) or non-protein molecule. For example, a mAb might be conjugated to a water soluble polymer such as polyethylene glycol or a carbon nanotube (See, e.g., Kam et al., Proc. Natl. Acad. Sci. USA 102: 11600-11605, 2005). See, U.S. patent application number 11/754,899.

[0030] Preferably, to ensure that high titers of human IL-1α -specific mAb can be administered to a subject with minimal adverse effects, the mAb compositions of the invention are at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.9 or more percent by weight pure (excluding any excipients). The mAb compositions of the disclosure might include only a single type of mAb (i.e., one produced from a single clonal B lymphocyte line) or might include a mixture of two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) different types of mAbs. In addition to human IL-1α mAbs, the Ab compositions of the disclosure might also include other mAbs that specifically bind antigens other than human IL-1α.

[0031] To modify or enhance their function, the human IL-1α mAbs might be conjugated another molecule such as a cytotoxin or detectable label. A human IL-1α specific mAb might be conjugated with one or more cytotoxins to more effectively kill cells expressing IL-1α. Cytotoxins for use in the disclosure can be any cytotoxic agent (e.g., molecule that can kill a cell after contacting the cell) that can be conjugated to a human IL-1α specific mAb. Examples of cytotoxins include, without limitation, radionuclides (e.g., ^{35}S, ^{14}C, ^{32}P, ^{125}I, ^{131}I, ^{90}Y, ^{89}Zr, ^{201}Tl, ^{186}Re, ^{188}Re, ^{57}Cu, ^{213}Bi, and ^{211}At), conjugated radionuclides, and chemotherapeutic agents. Further examples of cytotoxins include, but are not limited to, antimetabolites (e.g., 5-fluorouricil (5-FU), methotrexate (MTX), fludarabine, etc.), anti-microtubule agents (e.g., vincristine, vinblastine, colchicine, taxanes (such as paclitaxel and docetaxel), etc.), alkylating agents (e.g., cyclophasphamide, melphalan, bischloroethylnitrosurea (BCNU), etc.), platinum agents (e.g., cisplatin (also termed cDDP), carboplatin, oxaliplatin, JM-216, CI-973, etc.), anthracyclines (e.g., doxorubicin, daunorubicin, etc.), antibiotic agents (e.g., mitomycin-C), topoisomerase inhibitors (e.g., etoposide, tenoposide, and camptothecins), or other cytotoxic agents such as ricin, diptheria toxin (DT), Pseudomonas exotoxin (PE) A, PE40, abrin, saporin, pokeweed viral protein, ethidium bromide, glucocorticoid, anthrax toxin and others. See, e.g., U.S. Pat. No. 5,932,188.

[0032] The human IL-1α specific mAb can also be conjugated to a detectable label. Useful detectable labels in the present disclosure include biotin or streptavidin, magnetic beads, fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, or ^{72}As), radioopaque substances such as metals for radioimaging, paramagnetic agents for magnetic resonance imaging, enzymes (e.g., horseradish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters. Fluorescent markers may also be used and can be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

[0033] The present disclosure also encompasses nucleic acid molecules encoding fully human mAbs specific for human IL-1α. Although the same nucleic acid molecule might encode both the heavy and light chains of a human IL-1α -specific mAb, two different nucleic acid molecules, one encoding the heavy chain and the other encoding the light chain

might also be used. The amino acid sequences of three IgG1 mAbs specific for human IL-1α are presented herein. See SEQ ID NOs: 1, 3, 5, 7, 9, and 11. Exemplary nucleic acid molecules encoding these amino acid sequences are also described herein. See SEQ ID NOs: 2, 4, 6, 8, 10, and 12. Any other suitable nucleic acid that encodes the amino acid sequences of the two described IgG1 mAbs or other mAbs within the invention might also be used.

[0034] For production of mAbs, the nucleic acid molecules of the invention might be incorporated into an expression vector in an orientation wherein such nucleic acid molecules are operatively linked to expression control sequences such as transcriptional and translational control sequences. Examples of expression vectors include vectors derived from plasmids and vectors derived from viruses such as adenoviruses, adeno-associated viruses, and retroviruses. The nucleic acid molecules encoding a light chain and a heavy chain might be incorporated into a single vector or different vectors. The vectors of the disclosure might also include regulatory sequences such as promoters and/or enhancers (see, U.S. Pat. No. 5,168,062, U.S. Pat. No. 4,510,245 and U.S. Pat. No. 4,968,615), selectable markers, or sequences encoding affinity tags (for facilitating purification) or a detectable label.

[0035] For production of mAbs, the vectors of the disclosure can be introduced into a suitable host cell, e.g., a prokaryotic cell such as a bacteria or, preferably, a eukaryotic cell such as mammalian, plant, or yeast host cell. Examples of methods for introducing heterologous polynucleotides into host cells include use of viral vectors, electroporation, encapsulation of the polynucleotide(s) in liposomes, dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, Agrobacterium-mediated transformation, biolistic transformation, and direct microinjection of the DNA into nuclei. Mammalian cell lines are presently preferred for expression of mAbs from vectors. Examples of mammalian host cells include Chinese hamster ovary (CHO) cells (e.g., the DG44 CHO cell line), HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), NS0 cells, SP2 cells, HEK-293T cells, 293 Freestyle cells, and NIH-3T3 cells. The mAbs of the invention might also be expressed in transgenic animals or plants. See, e.g., U.S. Pat. Nos. 5,827,690; 5,756,687; 5,750,172; 5,741,957; 6,046,037; and 5,959,177.

[0036] The disclosure provides a method for detecting a human IL-1α-expressing cell in a sample by contacting the cell with a human IL-1α-specific mAb and detecting the mAb bound to the cell. The disclosure also provides a method for killing a human IL-1α-expressing cell by contacting the cell with a human IL-1α-specific mAb. Such killing can be accomplished by complement-mediated killing, Ab-dependent cell-mediated cytotoxicity, or Ab-mediated delivery of a cytotoxin. The Abs described herein have also been shown to be useful for other methods. For example, MABp1 has been to reduce IL-1α induced ICAM1 and E-selectin expression on endothelial cells. MABp1 has also been shown to be used in immunoassays for detecting and quantifying IL-1α in a biological sample.

EXAMPLES

Reference Example 1- Cloning of anti-hIL-la IgG1 and Kappa chains

[0037] Variable region heavy chain (V-HC) and variable region light chain (V-LC) sequences were gene synthesized using amino acid sequence information provided in US patent number 5,959,085. V-HC was PCR amplified introducing HindIII/ClaI sites upstream of the ATG start codon and a NheI site at the 3' end. The human germline IgG1 constant region (including exons and introns) was PCR amplified modifying the two 5' triplets encoding for the first two amino acids Ala-Ser to an NheI site, and introducing a BamHI site at the 3' end. The human germline IgG1 constant region amino acid sequence corresponded to Swiss-Prot entry P01857, except for a K171Q and a V261L exchange. The V-HC and constant IgG1-HC sequence were ligated using the NheI site and cloned into pcDNA3 using HindIII and BamHI sites.

>hIL-1a-IgG1-HC

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCTASGFTFSMFG
VHWVRQAPGKGLEWVAAVSYDGSNKYYAESVKGRFTISRDNSKNILFLQMD
SLRLEDTAVYYCARGRPKVVIPAPLAHWGQGTLVTFSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAQTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIALEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:1)

>hIL-1a-IgG1-HC

atggagttcgggctgagttgggtgttcctggtggctctgctgcggggcgtgcagtgccaggtgcagctggtggagagtgg
gggtggcgtggtgcagcctggccggtctctgcgcctgtcttgcactgcctccggttttacctttctatgtttggtgtgcactgg
gtgcgccaggctcccggcaagggactggaatgggtggccgccgtgagttacgacgggtccaacaaatattacgctgaga
gcgtgaaaggcagattcaccatcagcagagataattccaagaatattctgttcctgcagatggacagtctgagactggagg
acactgctgtgtactactgcgctcgtggacgccctaaggtggtcatccccgccccctggcacattggggccagggaactc
tggtgacctttctagcgctagcaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctgggggca
cagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccag
cggcgtccacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtagtgaccgtgccctccagcag
cttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaa
tcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaa
aacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctga
ggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcccagacaaagccgcgggaggagcagtacaacag
cacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccc
tgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
gccctggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcc

ttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccttaagtccgggaaaataa (SEQ ID NO:2)

[0038] The V-LC was PCR amplified introducing HindIII/ClaI sites upstream of the ATG start codon and a BsiWI site at the 3' end. The human constant Kappa-LC sequence was PCR amplified introducing a 5' BsiWI site encoding an additional Arg and the first amino acids Thr, and a BamHI site at the 3' end. The human constant Kappa-LC amino acid sequence corresponded to Swiss-Prot entry P01834. V-HC and constant Kappa-LC sequences were ligated using the BsiWI site and cloned into pcDNA3 using HindIII and BamHI sites.

>hIL-la-K-LC

MDMRVPAQLLGLLLLWFPGSRCDIQMTQSPSSVSASVGDRVTITCRASQGISS
WLAWYQQKPGKAPKLLIYEASNLETGVPSRFSGSGSGSDFTLTISSLQPEDFA
TYYCQQTSSFLLSFGGGTKVEHRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC [SEQ ID NO:3]

>hIL-la-K-LC

atggacatgcgcgtgcccgcccagctgctggggctgctgctgctgtggttccctggatctaggtgcgacattcagatgacc
cagtcccccagctcagtgtcagcctccgtgggcgacagagtgacaatcacctgccgcgcctctcagggaatctctagttgg
ctggcctggtaccagcagaagcctggaaaggcccccaagctgctgatctatgaagcctccaacctggagaccggcgtgc
cctctcgcttcagcggctcaggctcaggcagtgattttactctgaccatcagctccctgcagccagaggatttcgctacttact
actgccagcagacctcttccttcctgctgtccttcgggggaggcacaaaggtggagcaccgtacggtggctgcaccatctg
tcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagaga
ggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaa
ggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcga
agtcacccatcagggcctgagttcaccggtgacaaagagcttcaacaggggagagtgttag[SEQ ID NO:4]

Reference Example 2- Generation of NATHMAB-hIL-1a IgGI and Kappa chain

[0039] The complete sequence encoding the NATHMAB-hIL-1a/IgG1 heavy chain was gene synthesized. The V-HC sequence corresponded to the amino acid sequence described in US Patent 5,959,085. The human constant IgG1-HC sequence corresponded to Swiss-Prot entry P01857. The nucleotide sequence was codon optimized for expression in CHO cells. A Kozac sequence (gccacc) was added upstream of the start ATG.

>NATHMAB-hIL-1A-IGG1-HC

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCTASGFTFSMFG
VHWVRQAPGKGLEWVAAVSYDGSNKYYAESVKGRFTISRDNSKNILFLQMD
SLRLEDTAVYYCARGRPKVVIPAPLAHWGQGTLVTFSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
[SEQ ID NO:5]

>NATHMAB-hIL-1A-IGG1-HC

gccaccatggagtttggtctgtcctgggtgttcttggtggctctgctgaggggggtgcagtgccaggtccagctggtggagt
ctggtgggggagtggtgcagcctgggagatctctgcggctgtcttgcactgcctctggtttcactttctctatgtttggtgtgca
ttgggtcaggcaagcaccaggcaaaggactcgagtgggtcgcagctgtgagctatgacgggtctaacaaatattacgctg
agtctgtcaagggtaggtttaccatcagccgggataattccaaaaatatcctgttcctgcaaatggactctctgaggctggaa
gatactgcagtctactattgtgcaaggggggaggccaaaggtggtgatccccgctcccctcgctcactggggacagggaac
cctggtgactttcagctctgctagcaccaagggccctagcgtgttcccattggctccttcctccaaatctacttctggaggcac
cgccgccctgggatgtctcgtgaaagattattttcctgagcccgtcaccgtgagctggaacagcggcgccctgactagcgg
cgtgcacacctttcccgcagtgctgcaatctagcgggctgtactccctgagctctgtcgtgaccgtgccctccagcagcctc
ggaactcagacctacatctgcaatgtcaatcataaaccctctaataccaaagtcgataagaaggtcgaacctaaatcttgcga
taaaacccatacctgccccccttgcccagcacccgaactgctgggcggtccctctgtgtttctgttcccccccaaacccaaa
gataccctgatgatctctaggacccccgaggtcacttgtgtcgtggtggatgtgtcccacgaagatccagaagtcaaattca
actggtatgtggacggggtcgaagtgcacaacgcaaagaccaagcctagggaggaacagtataatagcacatatagggt
ggtcagcgtcctgaccgtcctgcatcaggactggctgaatggcaaagaatataagtgtaaagtgtccaacaaggccctgcc
agccccaatcgaaaagacaatctctaaagccaaggggcaaccccgggaacctcaggtctatacactgccacccctctcgg
gatgaactgaccaagaatcaggtgagcctgacatgtcttgtgaagggttttatccctccgacattgccgtggagtgggaga
gcaatggacaaccagaaaataactacaaaaccacacccccctgtgctggactccgatggttccttcttcctctactctaagctg

acagtggataagtctaggtggcagcaggggaatgtgttctcctgctctgtgatgcacgaggcactgcacaatcattatacac
aaaagtctctgtctctgtctccaggaaagtaa [SEQ ID NO:6]

[0040] The complete sequence encoding the NATHMAB-hIL-1a/Kappa light chain was gene synthesized. The V-LC sequence corresponded to the amino acid sequence described in US Patent 5,959,085. The human constant Kappa-

LC sequence corresponded to Swiss-Prot entry P01834. The nucleotide sequence was codon optimized for expression in CHO cells. A Kozac sequence (gccacc) was added upstream of ATG.

>NATHMAB-hIL-1A-K-LC

MDMRVPAQLLGLLLLWFPGSRCDIQMTQSPSSVSASVGDRVTITCRASQGISS
WLAWYQQKPGKAPKLLIYEASNLETGVPSRFSGSGSGSDFTLTISSLQPEDFA
TYYCQQTSSFLLSFGGGTKVEHTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC [SEQ ID NO:7]

NATHMAB-hIL-1A-K-LC

gccaccatggacatgcgcgttcctgcccagctcctcggactgctgctgctttggttcccaggctcccggtgtgatattcag
atgacacagtctccctcctccgtatctgcatccgtgggcgacagggtcacaatcacttgtagggccagccaggggatctc
tagttggctcgcatggtaccaacaaaagccaggtaaggctccgaaactgctcatttacgaagctagtaacctcgaaacag
gcgtgccaagccggtttagcggctccggttccggttctgacttcaccctcactatttcctccctgcaacctgaggattttgcc
acatattactgtcagcaaacttcttcttttctgctctcctttggtgggggaactaaggtggagcacacagtggccgccccca
gcgtctttatcttccccccaagcgatgaacagctgaagtcagggaccgccagcgtggtctgcctgctcaataatttttaccc
tcgcgaggctaaggtccaatggaaagtggataacgccctccagagcggtaactctcaggagtctgtcacagagcaaga
cagcaaggatagcacctattccctctccagcaccctgacactgtctaaggccgactacgagaaacacaaagtgtacgctt
gtgaggtgactcaccagggactgagtagccctgtgacaaaatctttcaataggggagaatgctga [SEQ ID
NO:8]

Reference Example 3 - Expression of NATHMAB-IL1-a (IgG1/k subtype)

[0041] NATHMAB-IL-1α was expressed and purified using a transient transfection method. Cell culture supernatant or protein G affinity purified Ab was subjected to further analysis as described below. Human embryonic kidney (HEK) 293T cells were cultured in DMEM containing 10% FCS, and transiently transfected using jetPEI reagent (Polyplus) according to manufacturer's protocol. Cells were seeded on 10 cm dishes ($3x10^6$ cells per 10 cm dish) 24h prior to transfection to reach approximately 50% density at the time point of transfection. 5 μg per dish of pcDNA3-anti-hIL-1α-IgG1-HC and a 2-fold molar excess of pcDNA3-anti-hIL-1α-Kappa were used for transfection. After recovery, medium was changed to DMEM containing 2% FCS (10 ml per dish) and Ab was collected for 5 to 6 days. The supernatant was collected, filtered, pH adjusted to 7.5 - 8, and stored at 4°C until further use.

[0042] Part of the supernatant (250 ml) was incubated with protein G sepharose (GE Healthcare) for 3 h at 4°C on a rotation wheel. Then, the protein G sepharose was loaded onto a gravity flow column and washed with PBS. Ab was eluted in 1 ml fractions using 100 mM glycine/150 mM NaCl into 100 μl Tris (pH 8), followed by dialysis with PBS containing 10% glycerol. The total protein concentration of each fraction was measured using the BCA Protein Detection Kit (Pierce). Correct size of heavy and light chains, and of the assembled native Ab was confirmed by SDS-PAGE.

[0043] Supernatant containing NATHMAB-hIL-1α purified Ab and Triton X-100 cell lysates of producer HEK 293T cells were tested for antigen binding in a radioimmunoassay (RIA) using ^{125}I-hIL-1α. Binding was assayed by absorption to protein G. All samples bound ^{125}I-hIL-1α with highest activity in the eluate. Binding of purified NATHMAB-hIL-1α in a concentration of 0.012% (half-max activity in RIA) to ^{125}I-hIL-1α was used for measuring the affinity coefficient. The Ka of NATHMAB-hIL-1a under these conditions was $3.03x10^{10}$ M^{-1}. Back calculation revealed an estimated concentration of approximately 30 μg/ml active anti-hIL-1α-IgG in the purified eluate.

[0044] Neutralizing activity of NATHMAB-hIL1α was tested in a bioassay using the murine EL4-6.1 subline which produces high levels of IL-2 when treated with murine or human IL-1α (Zubler et al., J. Immunol. 134:3662-3668, 1985).

The indicated concentrations of NATHMAB-hIL-1α (eluate) were incubated for 30 min at 37°C with various concentrations of recombinant hIL-1α (eBioscience) in a final volume of 100 µl/well in a 96-well culture plate (flat bottomed). Each point was carried out in triplicate and in culture medium (DMEM, 5% FCS). To each well were added 100 µl of a suspension of EL4-6.1 cells ($5x10^5$ cells/ml) in culture medium containing 0.2 µg/ml ionomycin. After incubation for 24 h at 37°C in a 5% CO_2 incubator, cell free supernatants were harvested and assayed for IL-2 concentrations using a commercially available ELISA (R&D Systems). The results showed that NATHMAB-IL-1α effectively neutralized hIL-1α-induced IL-2 secretion by EL-4 cells.

[0045] To test for neutralization of membrane-bound hIL-1α, the same EL-4 cell-based assay as described above was used with following modifications. Different concentrations of NATHMAB-hIL-1a (eluate) were incubated with various numbers of human activated monocytes. For monocyte preparation, PBMC were isolated from buffy coat using Ficoll-Paque centrifugation. Monocytes were allowed to adhere for 1.5 h at 37°C in RPMI on plastic dishes. Non-adherent lymphocytes were washed away to yield a nearly pure monocyte culture. Monocytes were cultured in RPMI containing Gin, Pyr, and 10% FCS for 24 h with LPS (1µg/ml) at 37°C in a 5% CO_2 incubator. Cells were detached with PBS/2 mM EDTA, carefully scraped from plates, and transferred into Falcon tubes. Cells were washed twice with PBS, resuspended in PBS/1% PFA and fixed for 10 min at 20°C. Cells were washed with glycine buffer (150 mM glycine, 75 mM NaCl, pH 7.4), then with culture medium and counted. The results showed that NATHMAB-hIL-1α effectively neutralized IL-2 secretion by EL-4 cells induced by membrane-bound hIL-1α. In an experiment similar to that described above, NATHMAB-hIL-1α was tested for neutralization of murine IL-1α. Indicated amounts of NATHMAB-hIL-1a supernatant were incubated with recombinant human (h) or murine (m) IL-1α (eBioscience). The supernatant containing the Ab neutralized human, but not murine, IL-1α.

Reference Example 4- Ab-Mediated Killing of Cancer Cells

[0046] Human peripheral blood mononuclear cells (PBMC) isolated from the buffy coat by standard Ficoll Paque preparation were incubated in either RPMI-1640 CM or RPMI-1640-CM containing rhIL-2 (30ng/ml, ebioscience) at 37°C and 5% CO2 overnight and used as effector cells (E). THP1 cells were used as the targets (T). The assay was carried out in 96-well plates with each point in triplicate. After $1x10^4$ targets that were incubated with different concentration of MABp1 for 15 mins, effector cells were added in an ET ratio of 25:1 and 50:1 to $1x10^4$ targets and incubated for another 4 hours. 75ul of assay volume were transferred to a new 96-well plate and cytotoxicity was assayed using the LDH cytotoxicity detection kit (Roche) according to manufacturer's protocol. % specific lysis= (mean experimental release-mean spontaneous release without antibody) x 100/(mean maximal release from targets-mean spontaneous release from targets) A. untreated PBMC were used as effector cells. B. rhIL-2-treated PBMC were used as effector cells. In both cases, increasing concentrations (1.25 to 20 ug/ml) of MABp1 resulted in increased target cell killing (up to about 90%) at both ET ratios.

Reference Example 5- Human anti-ILla specific mAb sequences.

[0047] The complete sequence encoding for another human anti-hIL-1aIgG_1/Kappa light chain specific for human IL1α (MABp1) was synthesized and expressed as described above. In the nucleic acids encoding the heavy and light chains, a Kozac sequence (gccacc) was added upstream of the start ATG.

Heavy Chain

[0048]

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCTASGFTFSMFG
VHWVRQAPGKGLEWVAAVSYDGSNKYYAESVKGRFTISRDNSKNILFLQMD
SLRLEDTAVYYCARGRPKVVIPAPLAHWGQGTLVTFSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
[SEQ ID NO:9]

gccaccatggagtttggtctgtcctgggtgttcttggtggctctgctgaggggggtgcagtgccaggtccagctggtggagt
ctggtgggggagtggtgcagcctgggagatctctgcggctgtcttgcactgcctctggtttcactttctctatgtttggtgtgca
ttgggtcaggcaagcaccaggcaaaggactcgagtgggtcgcagctgtgagctatgacgggtctaacaaatattacgctg
agtctgtcaagggtaggtttaccatcagccgggataattccaaaaatatcctgttcctgcaaatggactctctgaggctggaa
gatactgcagtctactattgtgcaaggggggaggccaaaggtggtgatccccgctcccctcgctcactggggacagggaac
cctggtgactttcagctctgctagcaccaagggccctagcgtgttcccattggctccttcctccaaatctacttctggaggcac
cgccgccctgggatgtctcgtgaaagattattttcctgagcccgtcaccgtgagctggaacagcggcgccctgactagcgg
cgtgcacaccttteccgcagtgctgcaatctagcgggctgtactccctgagctctgtcgtgaccgtgccctccagcagcctc
ggaactcagacctacatctgcaatgtcaatcataaaccctctaataccaaagtcgataagagggtcgaacctaaatcttgcg
ataaaacccatacctgccccccttgcccagcacccgaactgctgggcggtccctctgtgtttctgttcccccccaaacccaa
agataccctgatgatctctaggacccccgaggtcacttgtgtcgtggtggatgtgtcccacgaagatccagaagtcaaattc
aactggtatgtggacggggtcgaagtgcacaacgcaaagaccaagcctagggaggaacagtataatagcacatataggg
tggtcagcgtcctgaccgtcctgcatcaggactggctgaatggcaaagaatataagtgtaaagtgtccaacaaggccctgc
cagccccaatcgaaaagacaatctctaaagccaaggggcaaccccgggaacctcaggtctatacactgccaccctctcgg

gaggaaatgaccaagaatcaggtgagcctgacatgtcttgtgaagggtttttatccctccgacattgccgtggagtgggaga
gcaatggacaaccagaaaataactacaaaaccacacccctgtgctggactccgatggttccttcttcctctactctaagctg
acagtggataagtctaggtggcagcaggggaatgtgttctcctgctctgtgatgcacgaggcactgcacaatcattatacac
aaaagtctctgtctctgtctccaggaaagtaa [SEQ ID NO:10]

Light Chain

[0049]

MDMRVPAQLLGLLLLWFPGSRCDIQMTQSPSSVSASVGDRVTITCRASQGISS
WLAWYQQKPGKAPKLLIYEASNLETGVPSRFSGSGSGSDFTLTISSLQPEDFA
TYYCQQTSSFLLSFGGGTKVEHKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC [SEQ ID NO:11]

gccaccatggacatgcgcgttcctgcccagctcctcggactgctgctgctttggttcccaggctcccggtgtgatattcagat
gacacagtctccctcctccgtatctgcatccgtgggcgacagggtcacaatcacttgtagggccagccaggggatctctag
ttggctcgcatggtaccaacaaaagccaggtaaggctccgaaactgctcatttacgaagctagtaacctcgaaacaggcgt
gccaagccggtttagcggctccggttccggttctgacttcaccctcactatttcctccctgcaacctgaggattttgccacatat
tactgtcagcaaacttcttcttttctgctctcctttggtggaggaactaaggtggagcacaagcggacagttgctgctcctagc
gtctttatcttccctccaagcgatgaacagctgaagtcagggaccgccagcgtggtctgcctgctcaataatttttaccctcgc
gaggctaaggtccaatggaaagtggataacgccctccagagcggtaactctcaggagtctgtcacagagcaagacagca
aggatagcacctattccctctccagcaccctgacactgtctaaggccgactacgagaaacacaaagtgtacgcttgtgaggt
gactcaccagggactgagtagccctgtgacaaaatctttcaataggggagaatgctga [SEQ ID NO:12]

Reference Example 6 - MABp1 Binding Affinity

[0050] The binding affinity of purified MABp1 was determined using surface plasmon resonanace (SPR) on a BIAcore 2000 instrument (GE Health Sciences). A mouse monoclonal anti-human IgG (Fc) Ab was covalently immobilized on the flow cells of a CM5 sensor chip using a human Ab capture kit and amine coupling kit (GE Health Sciences). Immobilization levels of 8000 - 14000 RU would typically be achieved. After immobilization of the mouse anti-human IgG (Fc) capture Ab, three start-up cycles with HBS-EP running buffer (GE Health Sciences) and two start-up cycles with MABp1 were run to stabilize the CM5 surface and to remove any non-covalently bound Ab. For analysis, MABp1 Ab was diluted into HBS-EP running buffer to a final concentration of 1 μg/mL and immobilized to 700 RU on one flow cell of the CM5 sensor chip. Carrier-free human IL-1A cytokine (eBioscience, #34-8019) was serially diluted in HBS-EP running buffer

over a test range from 100 nM to 0.05 nM. Flow rate was 30 μl/min. Dissociation data for each cytokine dilution was recorded for 15 minutes. The CM5 surface was regenerated after each cycle using a single injection of 3 M $MgCl_2$ for 25 seconds at a flow rate of 30 μl/min. BiaEvaluation software and a Langmuir binding model was used to fit the data. The K_D for MABp1 was determined to be less than 2.0×10^{-10} M.

Reference Example 7 - MABp1 Inhibits Tumor Cell Invasion of a Basement Membrane Matrix.

[0051] Matrigel (BD), a basement membrane matrix, was thawed at 4°C overnight and the dilute (5mg/ml to 1 mg/ml) in serum free cold cell culture media. 100 ul of the diluted matrigel was placed into the upper chambers of a 24-well transwell (Costar) and the transwell was incubated at 37°C for at least 4 to 5 h for gelling. Tumor cells (MDA-MB-231 and THP-1) were harvested from tissue culture flasks by Trypsin/EDTA, washed with culture media, and resuspended in medium containing 1% FBS at a density of 1×10^6 cells/ml. The gelled matrigel was gently washed with warmed serum free-culture media, and 100 ul of the cell suspension was added in each well. The lower chamber of the transwell was filled with 600 ul of culture media, and the plates was incubated at 37°C for 12 to 24 h. The cells that did not invade the matrigel were gently scraped off the top of each transwell with a cotton swab. The transwells were then removed from the 24-well plates and stained with crystal violet after fixing the invaded cells with 70% ethanol or methanol. The invaded cells were counted under a light microscope. The percent of cells invading the matrigel was significantly inhibited in the presence of MABp1.

Reference Example 8 - MABp1 Blocks Increase in ICAM1 Expression in Endothelial Cells.

[0052] Human umbilical vein endothelial cells (HUVEC) (BD Biosciences) were seeded to 24-well plates at 5×10^5 per well in 1 mL of M-200 medium supplemented with low-serum growth supplement (Invitrogen). Cells were allowed to settle for 3-4 hours. Medium was aspirated and a fresh 1 mL of M-200 was added per well. MABp1 was added directly to cells @ 4.26 μg/mL, co-incubated for 15 minutes at room temperature, and then recombinant human IL-1α (rhIL1A, eBioscience) was added to a final concentration of 40 pg/mL. Positive control wells received the addition of IL-1α only. HUVEC cells in the absence of IL-1α or the absence of MABp1 served as negative controls. After 17-20 hours incubation at 37 °C, 5% CO_2, cells were lifted from the plates by a non-enzymatic treatment for 20 minutes using CellStripper reagent (Cellgro Mediatech) and then immediately assayed for CD54 (ICAM-1) expression using standard flow cytometry protocols. Staining buffer comprised Dulbecco's PBS supplemented with 2% heat-inactivated fetal bovine serum. PE-conjugated mouse anti-human CD54 (ICAM-1) mAb (eBioscience, clone HA58) or a PE-conjugated mouse IgGlk isotype control (eBiocience, #12-4714) were used per manufacturer's instructions to stain HUVEC cells in a 100 microliter staining volume for 20 minutes in the dark at room temperature. Two washes in staining buffer were subsequently performed and then samples were acquired on a FACSCalibur flow cytometer (BD Biosciences). Among several independent experiments (n=5) the upregulation of ICAM-1 adhesion molecules induced by rhIL1A on the surface of HUVEC cells was neutralized by MABp1 to baseline levels exhibited by the unstimulated HUVEC cells.

Reference Example 9 - MABp1 Blocks Increase in E-selectin Expression in Endothelial Cells.

[0053] Similar to its effects on ICAM-1 induction, MABp1-mediated neutralization of induction of CD62E (E-selectin) on HUVEC cells was also observed. This effect was most pronounced when HUVEC cells were stimulated not by soluble rhIL-1a but by membranous IL-1α anchored by glycosyl-phosphatidylinositol to the surface of DG44 CHO cells (GPI-IL1A cells). In this experiment, confluent cultures of HUVEC cells in 6-well plates were co-cultured overnight with 5×10^6 GPI-IL1A DG44 cells in M-200 medium, either alone, in the presence of 10 μg/mL MABp1, or in the presence of 10 μg/mL D5 isotype control Ab. After 17-20 hours, HUVEC monolayers were washed extensively with Dulbecco's PBS and then lifted by non-enzymatic treatment for 20 minutes with CellStripper reagent (Cellgro Mediatech) and then immediately assayed for CD62E (E-selectin) expression using standard flow-cytometry protocols. Staining buffer comprised Dulbecco's PBS supplemented with 2% heat-inactivated fetal bovine serum. PE-conjugated mouse anti-human CD62E mAb (eBioscience, clone P2H3) or a PE-conjugated mouse IgGlk isotype control (eBiocience, clone P3) were used per manufacturer's instructions to stain HUVEC cells in a 100 microliter staining volume for 20 minutes in the dark at room temperature. Two washes in staining buffer were subsequently performed and then samples were acquired on a FACSCalibur flow cytometer (BD Biosciences). Upregulated E-selectin expression on the surface of HUVEC cells induced by membranous GPI-IL-1a was neutralized by MABp1 to baseline levels exhibited by unstimulated HUVEC cells.

Reference Example 10 - MRC-5 bioassay for MABp1 potency (neutralization of rhIL1A)

[0054] The MRC-5 cell line, derived from fetal human lung fibroblasts, was obtained from the ATCC collection (CCL-171). The IL-1 neutralizing potency of MABp1 was assayed by measuring IL-1A induced release of IL-6 from MRC-5

cells. MRC-5 cells were seeded at 5 x 10^3 per well to a 96-well plate in 100 microliters of DMEM complete medium. Cells were cultured overnight at 37 °C in a humidified 5% CO_2 incubator. Confluent MRC-5 cells were subsequently cultured another 24 hours with 20 pg/mL of recombinant human IL-1A (rhIL1A, eBioscience) either alone or in the presence of increasing concentrations of MABp1. Negative control cells were not stimulated with rhIL1A. After the 24 hours, supernatants were collected and assayed for IL-6 release using and IL-6 ELISA kit from eBioscience. The IC_{50}, or concentration of MABp1 required to inhibit 50% of the maximal IL-6 release, was in the range of 0.001-0.01 μg/mL.

Reference Example 11 - MABp1 Identifies IL-1a+ Cells.

[0055] One hundred microliters of sodium heparin anti-coagulated whole blood was aliquoted to polystyrene FACS tubes. Samples were incubated at room temperature for 15 minutes with 1 mg of human IgG (protein-A purified) plus 2 ml of heat-inactivated fetal bovine serum to block Fc receptors. Primary Abs were then added to the sample: Either 1 mg of Alexa-488 labeled MABp1, 1 mg of FITC-labeled monoclonal anti-membrane human ILIA Ab (FAB200F, R&D Systems), or 1 mg of a murine isotype control (IC002F, R&D Systems). Primary Abs were incubated with sample for 30 minutes at room temperature in the dark. Sample erythrocytes were then lysed (BD Biosciences PharmLyse solution) at room temperature for 15 minutes, centrifuged at 300 x g for 5 minutes, and aspirated. Sample pellets were washed three times with 1 mL Hank's balanced salt solution (HBSS) containing 2% heat-inactivated fetal bovine serum. Sample was resuspended in 0.3 mL HBSS + 2% FBS and data was acquired on a FACSCalibur flow cytometer and analyzed using CellQuest software. Flow cytometric analysis of human PBMC using MABp1 showed that only 0.2% of PBMC were positive for IL-1α.

Reference Example 12 - MABp1 for Detecting and Tracking Infections and Inflammation.

[0056] Flow cytometric analysis (as in Example 11) of human PBMC using MABp1 showed a 3.6-fold increase in the percent of PBMC positive for IL-1α^+ in a subject with a sub-clinical infection compared to a normal control. Similarly, in a subject with an inflamed wisdom tooth, an increase in the percent of PBMC positive for IL-1α^+. A substantial decrease in the number of IL-1α^+ PBMC was observed from 14 to 45 days after removal of the wisdom tooth.

Example 13 - Immunoassay for Detecting and/or Quantifying IL-1α.

[0057] In general, very low levels of IL-1α are present in the plasma of human subjects. Because these levels are often beyond the detection threshold of conventional immunoassays, an ELISA with improved sensitivity was developed. In this ELISA, exogenous anti-IL-la Ab (e.g., MABp1) can be added to a biological sample being tested (e.g., human plasma) under conditions that allow the Ab to bind IL-1α in the sample. Because, it was observed that almost all IL-1α in human plasma samples exists already bound to endogenous anti-IL-la Ab, the latter step can often be omitted. The sample with IL-1a-Ab complexes is then applied to a filter (Amicon centrifugal device) with a molecular weight cutoff of about 100 kDa to separate the IL-1a-Ab complexes from molecules in the sample less than the molecular weight cutoff. In one experiment, this resulted in a 50-fold concentration. The processed sample (and dilutions thereof) was then added to wells of a microtiter plate coated with an anti-human IgG capture Ab (2 ug/ml mouse anti-human IgG, Fc-specific, Southern Biotech product code #9042-01). After allowing time to bind the IL-1a-Ab complexes in the sample, the wells were washed to remove non-binding material. A labeled anti-human IL-1α secondary Ab was then added to the wells (0.2 ug/ml biotin-conjugated monoclonal mouse anti-human IL-1A Ab, clone CRM6, eBioscience catalog # 13-7017). After allowing time to bind the IL-1α in the wells, the plate was washed and the amount of labeled anti-human IL-1α in each well was quantified as an indication of the concentration of IL-1α in the sample being tested.

[0058] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

[0059]

<110> XBiotech USA, Inc. Simard, John

<120> Interleukin-lalpha Antibodies and Methods of Use

<130> P51082EP1

<140>
<141> 2009-06-01

<150> US 61/057,586
<151> 2008-05-30

<150> US 61/121,391
<151> 2008-12-10

<150> US 61/178,350
<151> 2009-05-14

<160> 12

<170> PatentIn version 3.5

<210> 1
<211> 471
<212> PRT
<213> homo sapiens

<400> 1

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                  10                  15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
            20                  25                  30

Pro Gly Arg Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe
        35                  40                  45

Ser Met Phe Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ala Ala Val Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65                  70                  75                  80

Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                85                  90                  95

Ile Leu Phe Leu Gln Met Asp Ser Leu Arg Leu Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Gly Arg Pro Lys Val Val Ile Pro Ala Pro Leu
        115                 120                 125
```

```
Ala His Trp Gly Gln Gly Thr Leu Val Thr Phe Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
225                 230                 235                 240

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300

Gly Val Glu Val His Asn Ala Gln Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
    370                 375                 380
```

```
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385                 390                 395                 400

Ile Ala Leu Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
                405                 410                 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
            420                 425                 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
        435                 440                 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
    450                 455                 460

Leu Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 2
<211> 1416
<212> DNA
<213> Homo sapiens

<400> 2

atggagttcg ggctgagttg ggtgttcctg gtggctctgc tgcggggcgt gcagtgccag 60

gtgcagctgg tggagagtgg gggtggcgtg gtgcagcctg gccggtctct gcgcctgtct 120

tgcactgcct ccggttttac cttttctatg tttggtgtgc actgggtgcg ccaggctccc 180

ggcaagggac tggaatgggt ggccgccgtg agttacgacg ggtccaacaa atattacgct 240

gagagcgtga aaggcagatt caccatcagc agagataatt ccaagaatat tctgttcctg 300

cagatggaca gtctgagact ggaggacact gctgtgtact actgcgctcg tggacgccct 360

aaggtggtca tccccgcccc cctggcacat tggggccagg gaactctggt gacctttct 420

agcgctagca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct 480

gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 540

tcgtggaact caggcgccct gaccagcggc gtccacacct tcccggctgt cctacagtcc 600

tcaggactct actccctcag cagcgtagtg accgtgccct ccagcagctt gggcacccag 660

acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 720

cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga actcctgggg 780

ggaccgtcag tcttcctctt ccccccaaaa cccaaggaca ccctcatgat ctcccggacc 840

cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac 900

tggtacgtgg acggcgtgga ggtgcataat gcccagacaa agccgcggga ggagcagtac 960

aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 1020

aaggagtaca agtgcaaggt ctccaacaaa gccctcccag cccccatcga gaaaaccatc 1080

tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgccccc atcccgggat 1140

gagctgacca agaaccaggt cagcctgacc tgcctggtca aaggcttcta tcccagcgac 1200

atcgccctgg agtgggagag caatgggcag ccggagaaca actacaagac cacgcctccc 1260

gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg 1320

tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggctctgca caaccactac 1380

acgcagaaga gcctctcctt aagtccggga aaataa 1416

<210> 3
<211> 235
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                  10                  15

Phe Pro Gly Ser Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
        35                  40                  45

Gln Gly Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn Leu Glu Thr Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
            100                 105                 110

Thr Ser Ser Phe Leu Leu Ser Phe Gly Gly Gly Thr Lys Val Glu His
        115                 120                 125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
    130                 135                 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145                 150                 155                 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
                165                 170                 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
            180                 185                 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
        195                 200                 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
    210                 215                 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 4
<211> 708
<212> DNA

<213> Homo sapiens

<400> 4

```
atggacatgc gcgtgcccgc ccagctgctg gggctgctgc tgctgtggtt ccctggatct   60
aggtgcgaca ttcagatgac ccagtccccc agctcagtgt cagcctccgt gggcgacaga  120
gtgacaatca cctgccgcgc ctctcaggga atctctagtt ggctggcctg gtaccagcag  180
aagcctggaa aggcccccaa gctgctgatc tatgaagcct ccaacctgga gaccggcgtg  240
ccctctcgct tcagcggctc aggctcaggc agtgatttta ctctgaccat cagctccctg  300
cagccagagg atttcgctac ttactactgc cagcagacct cttccttcct gctgtccttc  360
gggggaggca caaaggtgga gcaccgtacg gtggctgcac catctgtctt catcttcccg  420
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc  480
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc  540
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg  600
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag  660
ggcctgagtt caccggtgac aaagagcttc aacaggggag agtgttag               708
```

<210> 5
<211> 471
<212> PRT
<213> Homo sapiens

<400> 5

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                  10                  15
```

```
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
            20                  25                  30

Pro Gly Arg Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe
        35                  40                  45

Ser Met Phe Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ala Ala Val Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65                  70                  75                  80

Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                85                  90                  95

Ile Leu Phe Leu Gln Met Asp Ser Leu Arg Leu Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Gly Arg Pro Lys Val Val Ile Pro Ala Pro Leu
        115                 120                 125

Ala His Trp Gly Gln Gly Thr Leu Val Thr Phe Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
225                 230                 235                 240

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270
```

```
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
    370                 375                 380

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385                 390                 395                 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
                405                 410                 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
            420                 425                 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
        435                 440                 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
    450                 455                 460

Leu Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 6
<211> 1422
<212> DNA
<213> Homo sapiens

<400> 6

gccaccatgg agtttggtct gtcctgggtg ttcttggtgg ctctgctgag gggggtgcag 60

tgccaggtcc agctggtgga gtctggtggg ggagtggtgc agcctgggag atctctgcgg 120

ctgtcttgca ctgcctctgg tttcactttc tctatgtttg gtgtgcattg ggtcaggcaa 180

gcaccaggca aaggactcga gtgggtcgca gctgtgagct atgacgggtc taacaaatat 240

tacgctgagt ctgtcaaggg taggtttacc atcagccggg ataattccaa aaatatcctg 300

ttcctgcaaa tggactctct gaggctggaa gatactgcag tctactattg tgcaaggggg 360

aggccaaagg tggtgatccc cgctcccctc gctcactggg gacagggaac cctggtgact 420

ttcagctctg ctagcaccaa gggccctagc gtgttcccat tggctccttc ctccaaatct 480

acttctggag gcaccgccgc cctgggatgt ctcgtgaaag attattttcc tgagcccgtc 540

accgtgagct ggaacagcgg cgccctgact agcggcgtgc acacctttcc cgcagtgctg 600

caatctagcg ggctgtactc cctgagctct gtcgtgaccg tgccctccag cagcctcgga 660

actcagacct acatctgcaa tgtcaatcat aaaccctcta ataccaaagt cgataagaag 720

gtcgaaccta aatcttgcga taaaacccat acctgccccc cttgcccagc acccgaactg 780

ctgggcggtc cctctgtgtt tctgttcccc cccaaaccca aagataccct gatgatctct 840

aggacccccg aggtcacttg tgtcgtggtg gatgtgtccc acgaagatcc agaagtcaaa 900

ttcaactggt atgtggacgg ggtcgaagtg cacaacgcaa agaccaagcc tagggaggaa 960

cagtataata gcacatatag ggtggtcagc gtcctgaccg tcctgcatca ggactggctg 1020

aatggcaaag aatataagtg taaagtgtcc aacaaggccc tgccagcccc aatcgaaaag 1080

acaatctcta aagccaaggg gcaaccccgg gaacctcagg tctatacact gccaccctct 1140

cgggatgaac tgaccaagaa tcaggtgagc ctgacatgtc ttgtgaaggg tttttatccc 1200

tccgacattg ccgtggagtg ggagagcaat ggacaaccag aaaataacta caaaaccaca 1260

ccccctgtgc tggactccga tggttccttc ttcctctact ctaagctgac agtggataag 1320

tctaggtggc agcaggggaa tgtgttctcc tgctctgtga tgcacgaggc actgcacaat 1380

cattatacac aaaagtctct gtctctgtct ccaggaaagt aa 1422

<210> 7
<211> 234
<212> PRT
<213> Homo sapiens

<400> 7

```
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                  10                  15

Phe Pro Gly Ser Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
        35                  40                  45

Gln Gly Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn Leu Glu Thr Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
            100                 105                 110

Thr Ser Ser Phe Leu Leu Ser Phe Gly Gly Gly Thr Lys Val Glu His
        115                 120                 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
    130                 135                 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145                 150                 155                 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
                165                 170                 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
            180                 185                 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
        195                 200                 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
    210                 215                 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230
```

<210> 8
<211> 711
<212> DNA

<213> Homo sapiens

<400> 8

gccaccatgg acatgcgcgt tcctgcccag ctcctcggac tgctgctgct ttggttccca 60

ggctcccggt gtgatattca gatgacacag tctccctcct ccgtatctgc atccgtgggc 120

gacagggtca caatcacttg tagggccagc caggggatct ctagttggct cgcatggtac 180

caacaaaagc caggtaaggc tccgaaactg ctcatttacg aagctagtaa cctcgaaaca 240

ggcgtgccaa gccggtttag cggctccggt tccggttctg acttcaccct cactatttcc 300

tccctgcaac ctgaggattt tgccacatat tactgtcagc aaacttcttc ttttctgctc 360

tcctttggtg ggggaactaa ggtggagcac acagtggccg cccccagcgt ctttatcttc 420

cccccaagcg atgaacagct gaagtcaggg accgccagcg tggtctgcct gctcaataat 480

ttttaccctc gcgaggctaa ggtccaatgg aaagtggata acgccctcca gagcggtaac 540

tctcaggagt ctgtcacaga gcaagacagc aaggatagca cctattccct ctccagcacc 600

ctgacactgt ctaaggccga ctacgagaaa cacaaagtgt acgcttgtga ggtgactcac 660

cagggactga gtagccctgt gacaaaatct ttcaataggg gagaatgctg a 711

<210> 9
<211> 471
<212> PRT
<213> Homo sapiens

<400> 9

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                  10                  15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
            20                  25                  30

Pro Gly Arg Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe
        35                  40                  45

Ser Met Phe Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ala Ala Val Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65                  70                  75                  80

Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                85                  90                  95

Ile Leu Phe Leu Gln Met Asp Ser Leu Arg Leu Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Gly Arg Pro Lys Val Val Ile Pro Ala Pro Leu
        115                 120                 125

Ala His Trp Gly Gln Gly Thr Leu Val Thr Phe Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160
```

```
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
225                 230                 235                 240

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
    370                 375                 380

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385                 390                 395                 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
```

405 410 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
420 425 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
435 440 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
450 455 460

Leu Ser Leu Ser Pro Gly Lys
465 470

<210> 10
<211> 1422
<212> DNA
<213> Homo sapiens

<400> 10

gccaccatgg agtttggtct gtcctgggtg ttcttggtgg ctctgctgag gggggtgcag 60

tgccaggtcc agctggtgga gtctggtggg ggagtggtgc agcctgggag atctctgcgg 120

ctgtcttgca ctgcctctgg tttcactttc tctatgtttg gtgtgcattg ggtcaggcaa 180

gcaccaggca aaggactcga gtgggtcgca gctgtgagct atgacgggtc taacaaatat 240

tacgctgagt ctgtcaaggg taggtttacc atcagccggg ataattccaa aaatatcctg 300

ttcctgcaaa tggactctct gaggctggaa gatactgcag tctactattg tgcaaggggg 360

aggccaaagg tggtgatccc cgctcccctc gctcactggg gacagggaac cctggtgact 420

ttcagctctg ctagcaccaa gggccctagc gtgttcccat tggctccttc ctccaaatct 480

acttctggag gcaccgccgc cctgggatgt ctcgtgaaag attattttcc tgagcccgtc 540

accgtgagct ggaacagcgg cgccctgact agcggcgtgc acacctttcc cgcagtgctg 600

caatctagcg ggctgtactc cctgagctct gtcgtgaccg tgccctccag cagcctcgga 660

actcagacct acatctgcaa tgtcaatcat aaaccctcta ataccaaagt cgataagagg 720

gtcgaaccta aatcttgcga taaaacccat acctgccccc cttgcccagc acccgaactg 780

ctgggcggtc cctctgtgtt tctgttcccc cccaaaccca aagataccct gatgatctct 840

aggacccccg aggtcacttg tgtcgtggtg gatgtgtccc acgaagatcc agaagtcaaa 900

ttcaactggt atgtggacgg ggtcgaagtg cacaacgcaa agaccaagcc tagggaggaa 960

cagtataata gcacatatag ggtggtcagc gtcctgaccg tcctgcatca ggactggctg 1020

aatggcaaag aatataagtg taaagtgtcc aacaaggccc tgccagcccc aatcgaaaag 1080

acaatctcta aagccaaggg gcaaccccgg gaacctcagg tctatacact gccaccctct 1140

cgggaggaaa tgaccaagaa tcaggtgagc ctgacatgtc ttgtgaaggg tttttatccc 1200

tccgacattg ccgtggagtg ggagagcaat ggacaaccag aaaataacta caaaaccaca 1260

ccccctgtgc tggactccga tggttccttc ttcctctact ctaagctgac agtggataag 1320

tctaggtggc agcaggggaa tgtgttctcc tgctctgtga tgcacgaggc actgcacaat 1380

cattatacac aaaagtctct gtctctgtct ccaggaaagt aa 1422

<210> 11
<211> 236
<212> PRT
<213> Homo sapiens

<400> 11

```
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                  10                  15

Phe Pro Gly Ser Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
        35                  40                  45

Gln Gly Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn Leu Glu Thr Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
            100                 105                 110

Thr Ser Ser Phe Leu Leu Ser Phe Gly Gly Gly Thr Lys Val Glu His
        115                 120                 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp

            180                 185                 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 12
<211> 717
<212> DNA
<213> Homo sapiens

<400> 12

gccaccatgg acatgcgcgt tcctgcccag ctcctcggac tgctgctgct ttggttccca 60

ggctcccggt gtgatattca gatgacacag tctccctcct ccgtatctgc atccgtgggc 120

gacagggtca caatcacttg tagggccagc caggggatct ctagttggct cgcatggtac 180

caacaaaagc caggtaaggc tccgaaactg ctcatttacg aagctagtaa cctcgaaaca 240

ggcgtgccaa gccggtttag cggctccggt tccggttctg acttcaccct cactatttcc 300

tccctgcaac ctgaggattt tgccacatat tactgtcagc aaacttcttc ttttctgctc 360

tcctttggtg gaggaactaa ggtggagcac aagcggacag ttgctgctcc tagcgtcttt 420

atcttccctc caagcgatga acagctgaag tcagggaccg ccagcgtggt ctgcctgctc 480

aataatttttt accctcgcga ggctaaggtc caatggaaag tggataacgc cctccagagc 540

ggtaactctc aggagtctgt cacagagcaa gacagcaagg atagcaccta ttccctctcc 600

agcaccctga cactgtctaa ggccgactac gagaaacaca aagtgtacgc ttgtgaggtg 660

actcaccagg gactgagtag ccctgtgaca aaatctttca ataggggaga atgctga 717

Claims

1. A method for quantifying the amount of IL-1α in a biological sample, the method comprising the steps of:

   (a) separating a biological sample obtained from a human subject using a size-exclusion filter that separates molecules according to molecular weight into a first fraction comprising intact IgG complexed with IL-1α and second fraction consisting of molecules less than 100 Kda, wherein the biological sample comprises intact IgG complexed with IL-1α, wherein the first fraction does not pass through the filter, and wherein the second fraction passes through the filter; and
   (b) quantifying the amount of IL-1α in the first fraction.

2. The method of claim 1 comprising the steps of:

   (a) separating a biological sample obtained from a human subject using a size-exclusion filter that separates molecules according to molecular weight into a first fraction comprising intact IgG complexed with IL-1α and second fraction consisting of molecules less than 100 Kda, wherein the biological sample comprises intact IgG complexed with IL-1α, wherein the first fraction does not pass through the filter, and wherein the second fraction passes through the filter;
   (b) adding the first fraction to a substrate comprising immobilized anti-human IgG antibodies under conditions that allow IgG in the first fraction to specifically bind the anti-human IgG antibodies immobilized on the substrate;
   (c) washing the substrate to remove material in the first fraction that does not specifically bind the immobilized anti-human IgG antibodies;
   (d) contacting the substrate washed in step (c) with an antibody that specifically binds human IL-1α under conditions that allows the antibody that specifically binds human IL-1α to specifically bind any human IL-1α bound to the substrate;
   (e) washing the substrate to remove any of the Ab that specifically binds human IL-1α that is not bound to the substrate; and

(f) quantifying the amount of Ab that specifically binds human IL-1α remaining bound to the substrate after step (e).

Patentansprüche

1. Verfahren zum Quantifizieren der Menge von IL-1α in einer biologischen Probe, wobei das Verfahren die folgenden Schritte umfasst:

(a) das Auftrennen einer von einem humanen Subjekt erhaltenen biologischen Probe unter Verwendung eines Größenausschlussfilters, der Moleküle gemäß dem Molekulargewicht in eine erste Fraktion, die intaktes IgG komplexiert mit IL-1α umfasst, und eine zweite Fraktion, die aus Molekülen von weniger als 100 Kda besteht, trennt, wobei die biologische Probe intaktes IgG komplexiert mit IL-1α umfasst, wobei die erste Fraktion den Filter nicht passiert und wobei die zweite Fraktion den Filter passiert, und
(b) Quantifizieren der Menge an IL-1α in der ersten Fraktion.

2. Verfahren nach Anspruch 1, welches die folgenden Schritte umfasst:

(a) das Auftrennen einer von einem humanen Subjekt erhaltenen biologischen Probe unter Verwendung eines Größenausschlussfilters, der Moleküle gemäß dem Molekulargewicht in eine erste Fraktion, die intaktes IgG komplexiert mit IL-1α umfasst, und eine zweite Fraktion, die aus Molekülen von weniger als 100 Kda besteht, trennt, wobei die biologische Probe intaktes IgG komplexiert mit IL-1α umfasst, wobei die erste Fraktion den Filter nicht passiert und wobei die zweite Fraktion den Filter passiert,
(b) das Zugeben der ersten Fraktion zu einem immobilisierte Anti-Mensch-IgG-Antikörper umfassenden Substrat unter Bedingungen, die es IgG in der ersten Fraktion erlauben, spezifisch an die auf dem Substrat immobilisierten Anti-Mensch-IgG-Antikörper zu binden,
(c) das Waschen des Substrats zum Entfernen von nicht spezifisch an die immobilisierten Anti-Mensch-IgG-Antikörper bindenden Materials in der ersten Fraktion,
(d) das Inkontaktbringen des in Schritt (c) gewaschenen Substrats mit einem humanes IL-1α spezifisch bindenden Antikörper unter Bedingungen, die es dem humanes IL-1α spezifisch bindenden Antikörper erlauben, an jegliches an das Substrat gebundene humane IL-1α spezifisch zu binden,
(e) das Waschen des Substrats zum Entfernen von nicht an das Substrat gebundenem, spezifisch an humanes IL-1α bindenden Ab und
(f) das Quantifizieren der nach Schritt (e) an das Substrat gebunden verbliebenen Menge des humanes IL-1α spezifisch bindenden Ab.

Revendications

1. Procédé de quantification de la quantité d'IL-la dans un échantillon biologique, le procédé comprenant les étapes de :

(a) séparation d'un échantillon biologique obtenu à partir d'un sujet humain au moyen d'un filtre d'exclusion de taille qui sépare les molécules en fonction du poids moléculaire en une première fraction comprenant IgG intact complexé avec IL-la et une deuxième fraction constituée de molécules inférieures à 100 Kda, dans lequel l'échantillon biologique comprend IgG intact complexé avec IL-la, dans lequel la première fraction ne traverse pas le filtre, et dans lequel la deuxième fraction traverse le filtre ; et
(b) quantification de la quantité d'IL-la dans la première fraction.

2. Procédé selon la revendication 1 comprenant les étapes de :

(a) séparation d'un échantillon biologique obtenu à partir d'un sujet humain au moyen d'un filtre d'exclusion de taille qui sépare des molécules en fonction du poids moléculaire en une première fraction comprenant IgG intact complexé avec IL-la et une deuxième fraction constituée de molécules inférieures à 100 Kda, dans lequel l'échantillon biologique comprend IgG intact complexé avec IL-la, dans lequel la première fraction ne traverse pas le filtre, et dans lequel la deuxième fraction traverse le filtre ;
(b) ajout de la première fraction à un substrat comprenant un anticorps anti-IgG humain immobilisé dans des conditions qui permettent aux IgG dans la première fraction de se lier spécifiquement à l'anticorps anti-IgG humain immobilisé sur le substrat ;

(c) lavage du substrat pour éliminer un matériau dans la première fraction qui ne se lie pas spécifiquement à l'anticorps anti-IgG humain immobilisé ;
(d) mise en contact du substrat lavé dans l'étape (c) avec un anticorps qui se lie spécifiquement à IL-1α humain dans des conditions qui permettent à l'anticorps qui se lie spécifiquement à IL-1α humain de se lier spécifiquement à IL-1α humain lié au substrat ;
(e) lavage du substrat pour éliminer l'un quelconque de l'anticorps qui se lie spécifiquement à IL-1α humain qui n'est pas lié au substrat ; et
(f) quantification de la quantité d'anticorps qui se lie spécifiquement à IL-1α humain restant lié au substrat après l'étape (e).

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5959085 A **[0003] [0037] [0039] [0040]**
- US 4634664 A **[0025]**
- US 754899 **[0027] [0029]**
- US 5693762 A **[0028]**
- US 5530101 A **[0028]**
- US 5585089 A **[0028]**
- US 5932188 A **[0031]**
- US 5168062 A **[0034]**
- US 4510245 A **[0034]**
- US 4968615 A **[0034]**
- US 5827690 A **[0035]**
- US 5756687 A **[0035]**
- US 5750172 A **[0035]**
- US 5741957 A **[0035]**
- US 6046037 A **[0035]**
- US 5959177 A **[0035]**
- US 61057586 B **[0059]**
- US 61121391 B **[0059]**
- US 61178350 B **[0059]**

Non-patent literature cited in the description

- **WOOD et al.** *Arthritis and Rheumatism,* 1985, vol. 28 (2), 853-862 **[0003]**
- **RIEGER et al.** Glossary of Genetics: Classical and Molecular. Springer-Verlag, 1991 **[0016]**
- **LEWIN.** Genes V. Oxford University Press, 1994 **[0016]**
- Current Protocols in Immunology. John Wiley & Sons **[0023]**
- Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 2001, vol. 1-3 **[0023]**
- Current Protocols in Molecular Biology. Greene Publishing **[0023]**
- Handbook of Therapeutic Abs. Wiley-VCH, 2007 **[0023]**
- **R LAN FRESHNEY.** Culture of Animal Cells: A Manual of Basic Technique. Wiley-Liss, 2000 **[0023]**
- **MAUREEN A HARRISON ; LAN F RAE.** General Techniques of Cell Culture. Cambridge University Press, 1994 **[0023]**
- Guide to Protein Purification: Methods in Enzymology. Academic Press, 1990, vol. 182 **[0023]**
- **GODING.** Monoclonal Abs: Principles and Practice. Academic Press, 1986, 59-103 **[0026]**
- **E.G., MORRISON et al.** *Proc. Nat'l. Acad. Sci. USA,* 1984, vol. 81, 6851 **[0028]**
- **NEUBERGER et al.** *Nature,* 1984, vol. 312, 604 **[0028]**
- **TAKEDA et al.** *Nature,* 1984, vol. 314, 452 **[0028]**
- **MARKS et al.** *Bio/Technology,* 1992, vol. 10, 779-783 **[0029]**
- **BARBAS et al.** *Proc Nat. Acad. Sci. USA,* 1994, vol. 91, 3809-3813 **[0029]**
- **SCHIER et al.** *Gene,* 1995, vol. 169, 147-155 **[0029]**
- **YELTON et al.** *J. Immunol.,* 1995, vol. 155, 1994-2004 **[0029]**
- **JACKSON et al.** *J. Immunol.,* 1995, vol. 154 (7), 3310-9 **[0029]**
- **HAWKINS et al.** *J. Mol. Biol.,* 1992, vol. 226, 889-896 **[0029]**
- **KAM et al.** *Proc. Natl. Acad. Sci. USA,* 2005, vol. 102, 11600-11605 **[0029]**
- **ZUBLER et al.** *J. Immunol.,* 1985, vol. 134, 3662-3668 **[0044]**